

A Study on Correlation between Serum Ionized Calcium and Severity of Dengue Infection

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Abstract: Dengue fever is a major worldwide cause of morbidity and mortality. Serum free calcium (Ca²⁺) plays an important role in cardiac and circulatory function. We evaluated the correlation between severity of dengue and the serum free calcium.

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I. Introduction

Dengue is endemic in over 100 countries. Infection with the dengue virus may be asymptomatic or may give rise to a spectrum of clinical illness, ranging from undifferentiated fever to a severe life threatening hemorrhagic/shock syndrome with multiple organ failure and fatality. Early recognition of dengue with prompt and appropriate treatment is vital to limit morbidity and mortality. Recent guidelines, which have built on the wealth of experience in managing these patients over recent years have made management strategies more well defined. In brief, the mainstay of management of dengue is based on careful fluid management and monitoring, together with provision of organ support and correction of metabolic derangement where necessary. No specific treatment is available, although steroids and immunoglobulins have been used in the past with little evidence-based benefit. Hypocalcemia has been documented in dengue infection and is seen more frequently in patients with severe dengue. Nonetheless, evidence with regards to the role of calcium homeostasis in dengue is limited, and current recommendations do not mention the need to monitor or correct blood calcium levels in dengue. In this study, we tried to analyze the incidence of hypocalcemia in dengue fever by correlating the levels of serum ionized calcium with the severity of dengue infection in hospitalized patients.

II. Material And Methods

This cross-sectional study was done at A.J Institute of Medical Sciences in Mangalore, Karnataka. All probable cases of dengue were diagnosed using NS1 antigen and/or IgM antibody tests and were classified according to WHO criteria. All diagnosed & confirmed Dengue Fever patients who came to A J hospital, met a predefined inclusion and exclusion criteria initiated after obtaining clearance from the institutions ethical committee. The data was collected in pre-prepared proforma and then transferred to a master chart for analysis.

The significance of the differences between proportions (%) and means were tested using the z-test and student's t-test or ANOVA, respectively.

Data were analysed using SPSS version 22 statistical software package. A 'p' value less than 0.05 (p<0.05) is considered significant.

Method of collection of data: : All diagnosed & confirmed dengue fever patients who were admitted to A J hospital.

Study design: Cross sectional study

Study duration: 2 years

Sample size: 50

Subjects & selection method: A cross-sectional study was performed at A.J Institute of Medical Sciences, Mangalore after obtaining ethical clearance from ethical committee, AJIMS for a period of 24 months. Inpatients with confirmed dengue infection were recruited for the study, after written consent was obtained

Inclusion criteria:

1. All the patients admitted to AJ Hospital fulfilling the WHO 2009 criteria for SDI.
2. Written informed consent.

Exclusion criteria:

1. Oral supplemental calcium or vit.d intake
2. Malabsorption syndromes
3. Renal dysfunction
4. Drugs causing hypocalcemia eg.rifampicin bisphosphonates phenobarbitone phenytoin steroids chloroquine calcitonin
5. Those who do not give consent
6. Patients with hypertension, diabetes, cardiac and liver diseases and those on anti-hypertensive/anti-arrhythmic medications, calcium supplements, or any other drugs affecting calcium homeostasis were excluded, as these would alter the blood pressure, platelet count, liver enzymes and serum calcium levels.

Procedure methodology

Details of the patient and presenting complaints like fever duration, headache, retro-orbital pain, arthralgia, myalgia, rash, bleeding manifestations, altered sensorium, restlessness etc. were collected interviewer administered questionnaire. The clinical parameters recorded were pertaining to evidence of hemodynamic instability(pulse volume, blood pressure, pulse pressure, presence of cold extremities) evidence of fluid leakage (pleural effusion and ascitis).

In addition, the following investigations were performed in first 24 hours of admission: Haemoglobin, Total leucocyte count, platelet count, packed cell volume, Renal function tests - urea, creatinine, liver function tests - S.Bilirubin, S.Albumin, Aspartate aminotransferase (AST), Alanine aminotransferase (ALT), total calcium and serum free calcium levels.

Hypocalcemia was defined as serum free calcium level of <4.64mg/dl. Blood was collected in an EDTA tube for haemoglobin, total leucocyte count, platelet count and packed cell volume and analysed by automated analyser. For other investigations blood was collected in plain tubes. Blood for serum ionised calcium was collected without applying tourniquet. Roche 9180 Electrolyte Analyser for serum free calcium measurement.

Statistical analysis

The collected data was analysed using SPSS version 22 statistical software package. The significance of the differences between proportions (%) and means were tested using the z-test and student's t-test or ANOVA, respectively. In addition, Bonferroni's test was used to conduct post hoc analysis for the categorical columns.

A 'p' value less than 0.05 ($p < 0.05$) is considered significant.

Charts and tables were generated with the help of Microsoft Excel.

III. Result

A total of 50 patients with Dengue Fever were analyzed. The mean age of the study participants was 28.7 years (range 6–56 years). The majority 25(50%) of the study subjects were in the age group 21-30 years. The majority of the patients were males ($n = 39, 78\%$). The percentages for patients with severe dengue and dengue with and without WS were 24%, 40% and 36% respectively. 74% of the patients were diagnosed with dengue fever using Dengue NS1 test while 26% were diagnosed using Dengue IgM titre.

All patients were subsequently recovered and no deaths were reported among the study population. Majority ($n=48, 96\%$) of patients were without a previous history of dengue infections, while only 2 patients had a previous documented history of dengue infection.

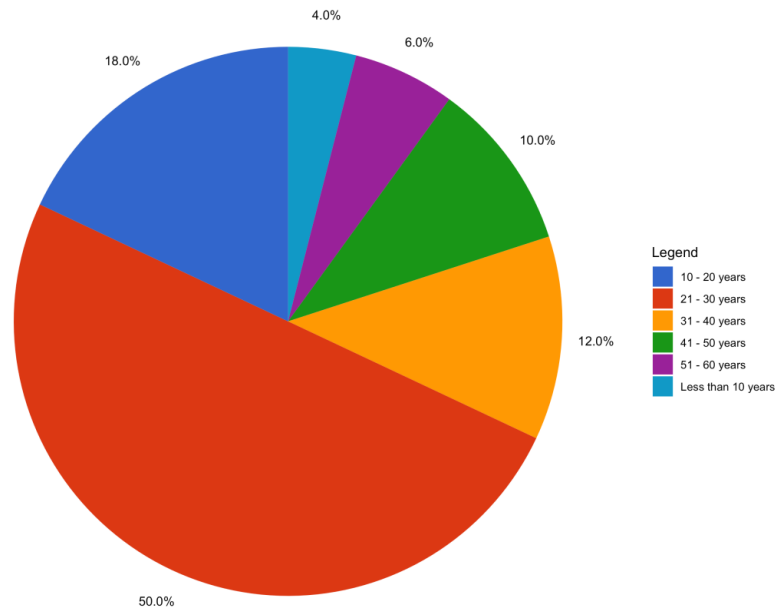
Serum ionized calcium level was measured during the first 24 hours of admission. Among the 50 patients, 33 (66%) showed hypocalcemia i.e. serum ionized calcium level was below 4.64 ,mg/dl considered as hypocalcemia. Mean calcium level of the population was 4.42 mg/dL, range being 4.12-4.68 mg/dL.

Free calcium was correlated with WHO class and the severity significantly correlated with the free calcium levels .Mean serum Ca^{2+} was significantly lower in patients with severe dengue (4.16mg/dl) than in those with dengue fever without warning signs and with warning signs (4.65mg/dl, 4.36mg/dl) ($p < 0.00001$).

Association was also found between free calcium and individual parameters like platelet count, ALT and haematocrit.

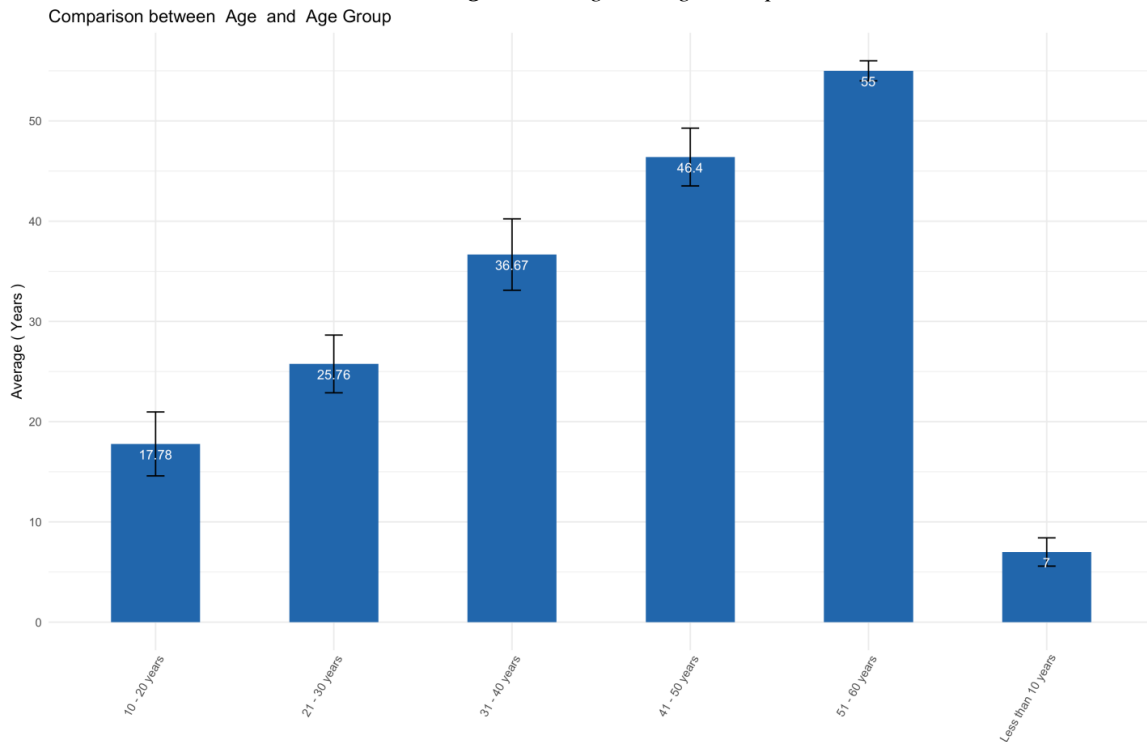
Pie Chart 1

Summary of Age Group

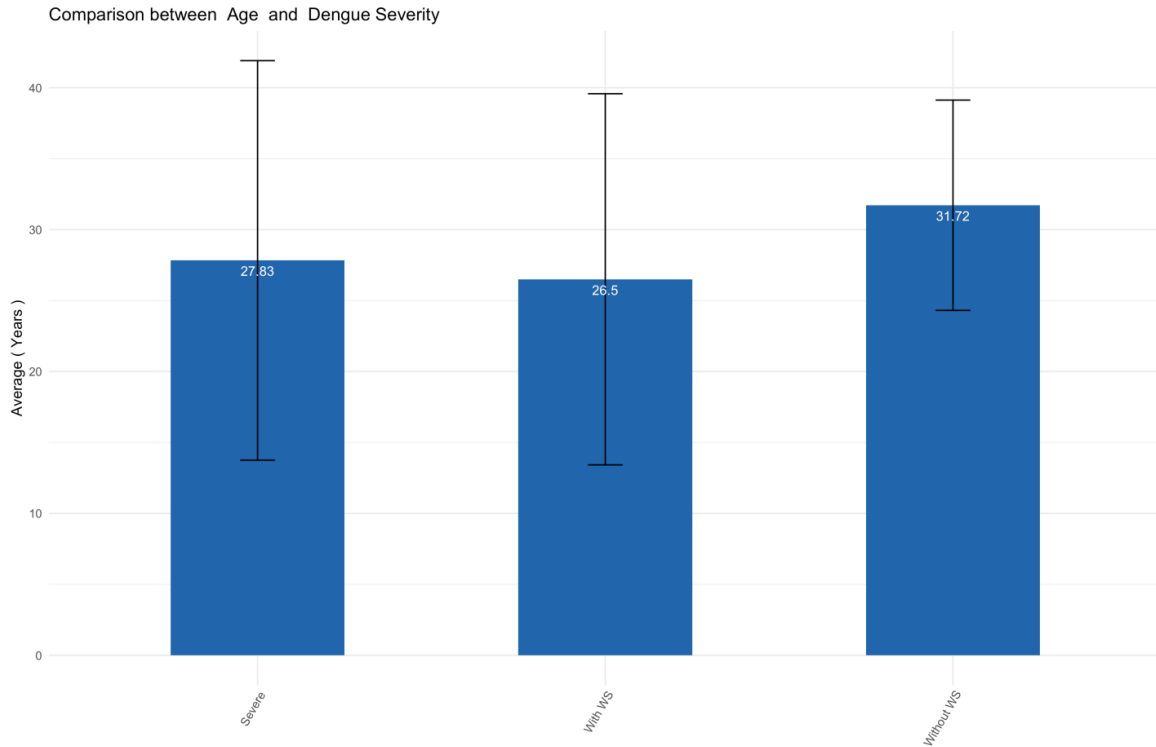


Age Group Distribution of Dengue Patients: Majority (50%), in 21-30 age bracket

Bar Diagram 1 – Age Vs. Age Group

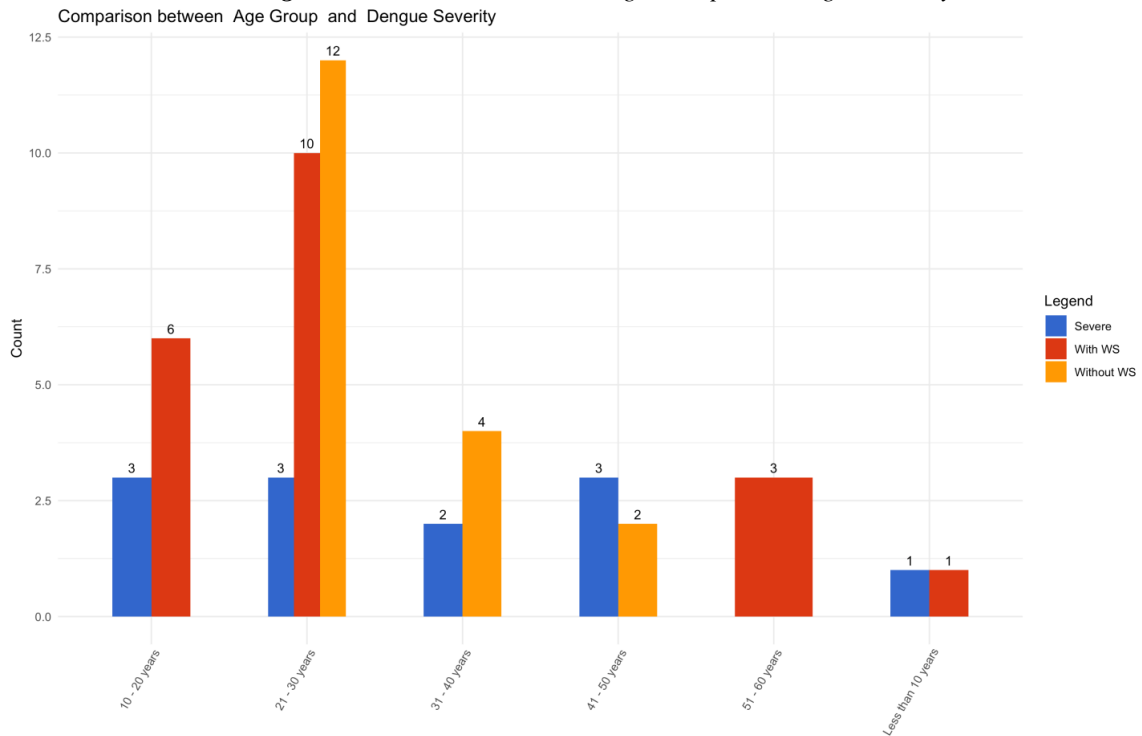


Bar Diagram 2 – Incidence of Dengue Severity in Different Ages

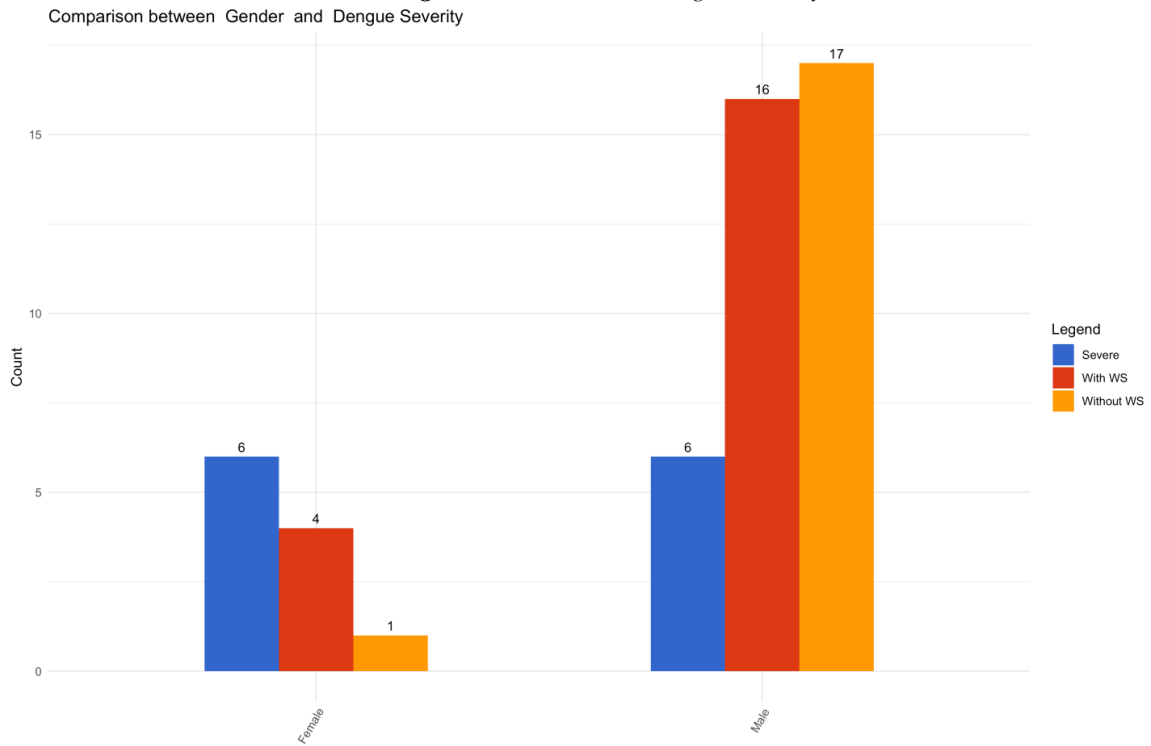


The severity of dengue fever measured according to WHO 2009 Dengue Guidelines. The average ages for:-
 Severe Dengue - 27.83 years
 Dengue with Warning Signs - 26.5 years
 Dengue without Warning Signs - 31.72 years

Bar Diagram 3– Correlation between Age Group and Dengue Severity

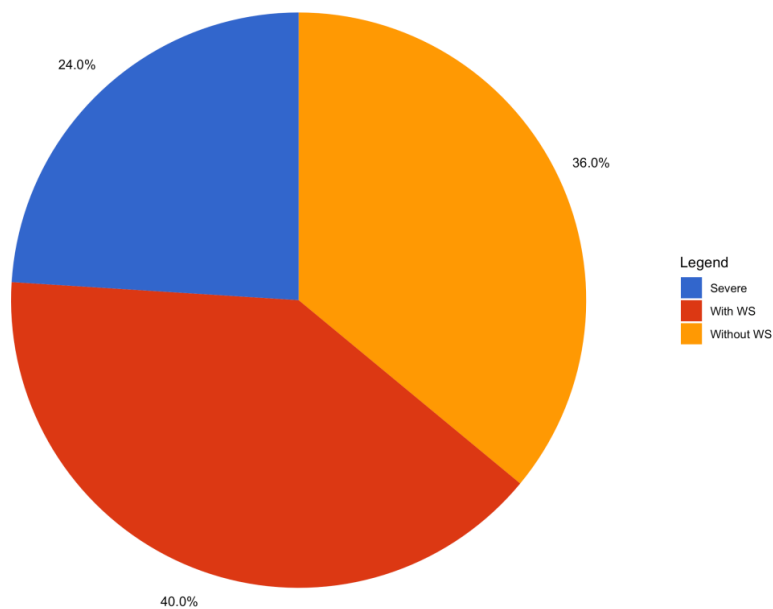


Bar Diagram 4 - Gender Vs. Dengue Severity



Pie Chart 2 -Spectrum of Dengue Severity

Summary of Dengue Severity



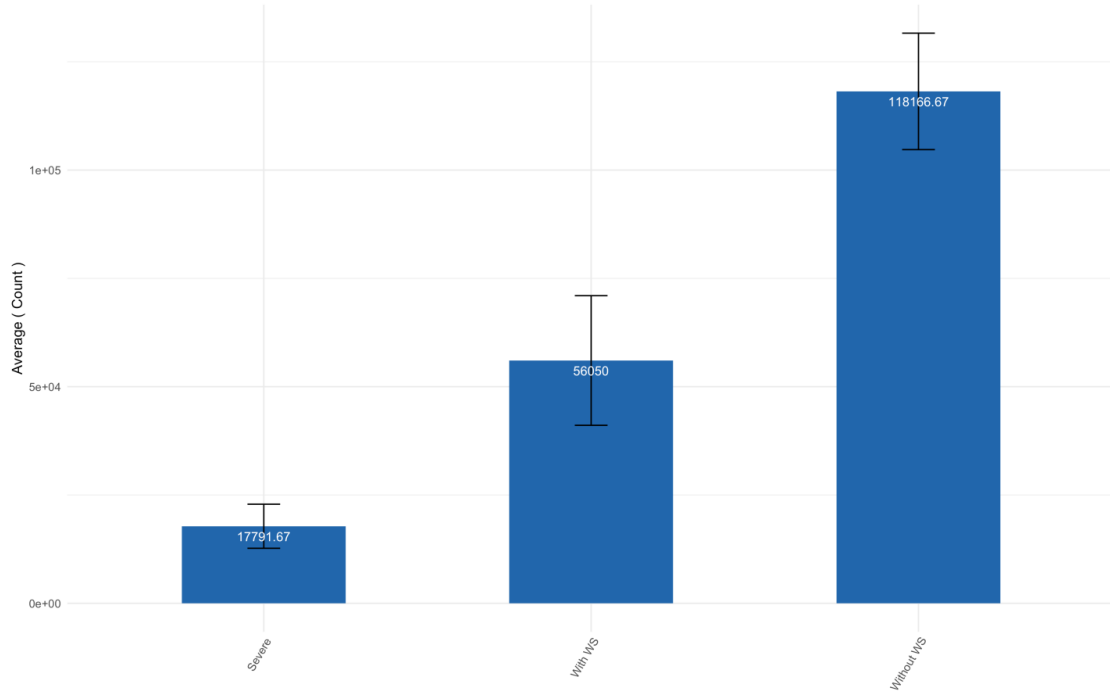
Severe Dengue – 24%

Dengue with Warning Signs – 40%

Dengue without Warning Signs – 36%

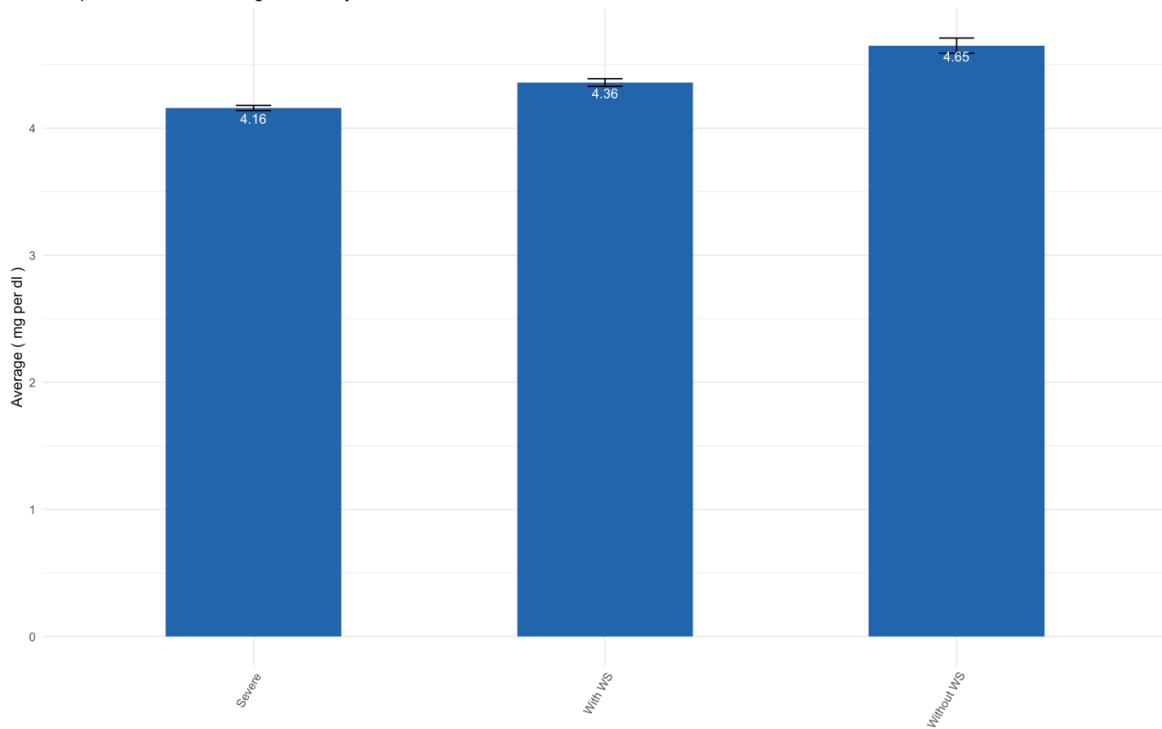
Bar Diagram 5 -Dengue Severity Vs. Platelets on Admission

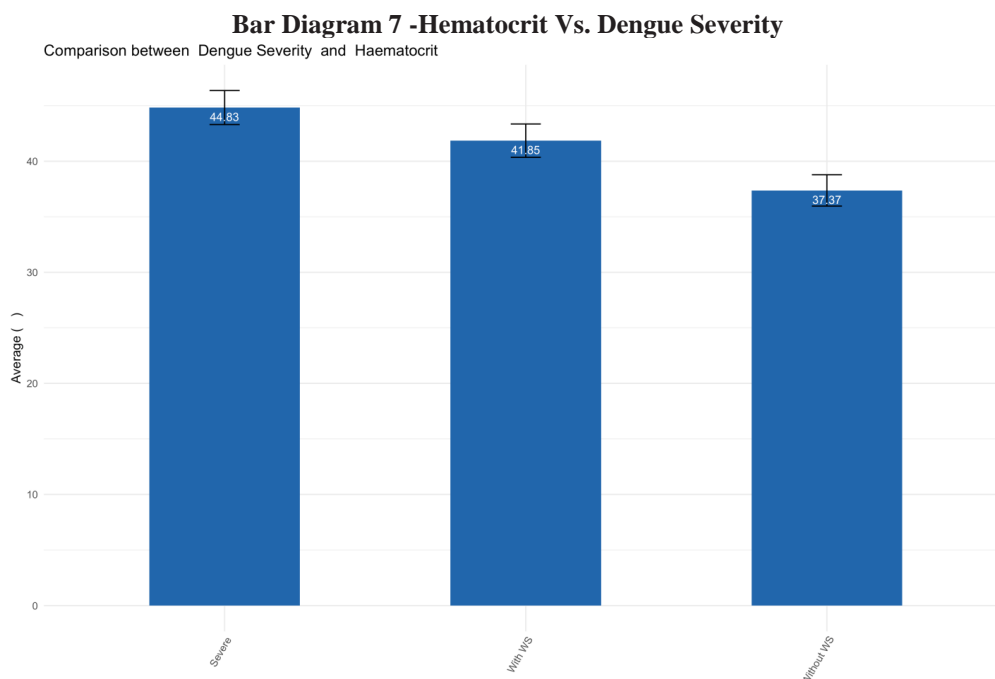
Comparison between Dengue Severity and Platelets on Admission



Bar Diagram 6 -Correlation between S.Ionized Calcium and Dengue Severity

Comparison between Dengue Severity and S Ionized Ca2





- The **mean age** of the study participants is 28.7 years(range 6-56 years).The minimum age is 6 years and the maximum age is 56 years.
- Majority, 25 (50%) of the study subjects were in the age group of 21-30 years.
- Majority of the individuals in the study were **males** (78%).
- It was observed that there was a statistically significant association(p value- 0.03415) between age and dengue severity
- There was a positive correlation(Correlation Coefficient-0.28) between age and serum ionized calcium with a p value- 0.046209
- However,there was NO statistically significant association between age vs. platelets on admission(p value- 0.111314) and age vs.hematocrit(p value-0.067729).
- A statistically significant association was found between different age groups and platelets on admission(p value- 0.046053)
- There was a statistically significant difference observed between the two genders when compared with platelet count(p value- 0.019776)
- A statistically significant difference was observed between the two genders vs.serum ionized calcium(p value- 0.010898)
- There was a statistically significant difference between males and females and the associated hematocrit levels(p value- 0.004103)
- It was observed that there was a statistically significant association(p value- <0.000001) between platelets on admission and dengue severity.
- A statistically significant association was found between serum ionized calcium and dengue severity(p value- <0.000001)
- It was observed that there was a statistically significant association(p value- <0.000001) between hematocrit and dengue severity.
- A statistically significant association was found between ALT levels and dengue severity(p value- <0.000001)
- It was observed that there was a statistically significant association(p value- <0.000001) between platelet counts on admission and serum ionized calcium
- A statistically significant association was found between between platelet counts on admission and hematocrit (p value- <0.000001)
- A statistically significant association was found between between platelet counts on admission and ALT levels(p value- <0.000001)
- It was observed that there was a statistically significant association(p value- <0.000001) between serum ionized calcium and hematocrit(p value- <0.000001)
- A statistically significant association was found between between serum ionized calcium and ALT levels(p value- <0.000001)

- However, there was NO statistically significant association between different age groups vs. serum ionized calcium (p value- 0.142281)

IV. Discussion

Dengue is the most prevalent mosquito-borne viral infection in the world ^[1]. Each year, there are ~50 million dengue infections and ~500,000 individuals are hospitalized with DHF, mainly in Southeast Asia, the Pacific, and the Americas ^[2]. 1.3 billion of these at-risk individuals live in ten dengue endemic countries in South East Asia ^[3]. Dengue is a rapidly emerging disease in India and has been prevalent for almost 230 years. India faced a major outbreak in 2015 and recorded 99913 cases and 220 deaths according to the National Vector Borne Disease Control Programme ^[4]. Rapid triage and effective therapy is very important in management of dengue. There is a need for newer modalities of treatment as the atypical manifestations with severe multi-organ involvement are increasingly being reported. Assessment of prognosis is also very important.

The spectrum of dengue infection varies from dengue fever without warning signs, to dengue with warning signs and severe dengue. In severe dengue infection numerous serum biochemical ^[5] parameter changes occur with the onset of plasma leakage.

Analyzing the relation between serum free calcium and its association with severe dengue infection may justify it to be utilized as a biochemical marker to differentiate severe dengue from nonsevere cases. Hypocalcaemia is known to be associated with plasma leakage during the critical phase of severe dengue, but limited studies are available about the presence of hypocalcaemia in severe dengue.

In a study by NJ Dahanayaka et al it was shown that low ionized calcium levels were a marker of plasma leakage and that hypocalcemia was usually transient. ^[6]

In view of the above said we did a study titled "A STUDY ON CORRELATION BETWEEN SERUM IONIZED CALCIUM AND SEVERITY OF DENGUE INFECTION" to study and assess the correlation between serum ionized calcium as a biochemical marker of severity of dengue infection. In this study it was noted that serum ionized calcium levels significantly correlated with the severity of dengue. In our study ionised calcium was low in 66% of patients. Mean calcium level of the above study population was 4.42 mg/dl significantly below the cut off mark for hypocalcemia, 4.64 mg/dl. Mean free calcium was found to be lower with increasing severity.

Several causes for low blood calcium levels have been suggested, including reduced Na⁺-K adenosine triphosphatase (ATPase) activity, reduced Ca²⁺-ATPase activity, acquired parathyroid hormone deficiency, renal one-alpha hydroxylase insufficiency, reduced dietary vitamin D intake, and reduced dietary calcium intake ^[7]. Ca²⁺ appears to play a role in the induction of dengue-specific T-helper cells. Dengue antigen has been shown to increase the influx of Ca²⁺ into T-cells. The proliferation of dengue-specific T-helper cells appears to be dependent on Ca²⁺ and is inhibited in the absence of Ca²⁺ and by calcium channel antagonist drugs ^[8]. Though there have been many studies on low serum calcium levels in sepsis, the role of calcium in dengue has not been elucidated completely. The exact mechanisms for development of hypocalcemia in severe dengue infections also requires further study.

Uddin et al. studied with 84 dengue patients demonstrated that hypocalcemia is an important biochemical derangement which is correlated with severity of dengue infection and it also revealed that mean serum calcium levels were within the normal range in non-severe dengue patients ^[9]. Another review endorsed the relationship between hypocalcemia and SDI, however monitoring serum calcium or calcium supplementation is not recommended in the current dengue management guidelines ^[10,11]. Considering above facts it is apparent that serum ionized calcium could be a potential biochemical marker in order to differentiate SDI from other dengue patients and plan out appropriate management in the clinical setting.

The study by Adikari et al. ^[12] concluded that serum ionized calcium level was significantly reduced in majority of patients with severe dengue infection within first 24 hours of onset of severe dengue clinical criteria. This was similar to the results of our study.

The current study has shown a relationship between serum free calcium and severe dengue infection. The mean serum free calcium was significantly lower in patients with severe dengue than in those with dengue fever without warning signs. A vast majority of deaths in dengue infections occur due to severe plasma leakage that occurs in DHF/DSS ^[1]. Therefore, the association between hypocalcemia and the severity of dengue needs to be further evaluated. Further studies with larger cohorts are needed to further evaluate the relationship between serum free calcium and severe dengue infection and can be used as a prognostic marker for dengue infection. The measurement of serum calcium is currently not a routine practice in patients with dengue infection.

Further studies are required to determine whether the presence of hypocalcemia at the onset of the illness can be utilized as a prognostic indicator to predict disease severity.

There have not been many studies to assess the effects of calcium supplementation for dengue patients. A study conducted in Mexico by Sanchez-Valdez et al. ^[13] on five patients with dengue infection demonstrated

that oral calcium carbonate and vitamin D3 supplementation significantly increased the number of platelets in patients with dengue infection when compared with a control group. A study done in Pune compared the levels of Vitamin D in patients having dengue infection with healthy individuals and found that it was significantly higher in patients with dengue^[14]. However, randomized control trials are presently not there to demonstrate the effectiveness of calcium therapy in the prevention of complications in dengue infection. Hence, oral or IV calcium therapy is not routinely included in published guidelines. Furthermore, hypocalcemia has also been demonstrated in certain cases of malaria, severe meningococcal infections, and other severe acute illnesses, being associated with a poor prognosis^[15-17].

Other biomarkers for severe dengue like Interleukins have been studied. Malavige et al^[18] studied serum Interleukin-10 (IL-10) as a marker of SDI but concluded as unsuitable to be used as a robust biomarker because of its poor discriminatory value between severe dengue and non-severe dengue patients.

Considering above facts, it is clear that serum ionized calcium could be a potential biochemical marker in order to identify severe dengue patients and plan appropriate management in the clinical setting. The pattern and trend of serum calcium in dengue patients can be revealed by further research and monitoring. This will be of greater importance considering the fact that the relatively lower cost of the serum calcium test and less operator or interpreter dependant than other imaging modalities used to diagnose severe dengue cases such as ultrasound scan^[19]. It is also important to study the clinical significance of hypocalcaemia in severe dengue and value of calcium supplementation in such patients with severe hypocalcaemia.

V. Conclusion

We conclude that the serum free calcium levels significantly correlated with the severity of DF. The serum free calcium levels were significantly lower in patients with Severe Dengue and Dengue fever with Warning Signs than in those with Dengue Fever. Free calcium can be used as a prognostic marker for dengue severity but further studies are required to support this.

In addition, more trials are needed to analyse the effect of supplementation of calcium in dengue fever for reduction of severity.

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